IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re application of:
SIMMS, Domenica A.
Appl. No. 09/058,350
Filed: April 10, 1998
For: **RNA Isolation Reagent and Methods**

Confirmation No.:
Art Unit: 1623
Examiner: Owens, H.
Atty. Docket: 0942.3840001/RWE/LBB

Brief on Appeal to the Board of Patent Appeals and Interferences Under 37 C.F.R. § 1.192

Commissioner for Patents
Washington, D.C. 20231

Sir:

A Notice of Appeal from the final rejection of claims 2-11 and 13-34 for the above-captioned U.S. Patent Application was filed on September 26, 2001. Appellant hereby files an appeal brief in triplicate as required under 37 C.F.R. § 1.192(a). Appellant has also filed herewith the fee for filing a brief in support of an appeal as set forth in 37 C.F.R. § 1.17(f), a petition for a five-month extension of time and the appropriate fee for said extension.

I. Real Party in Interest (37 C.F.R. § 1.192(c)(1))

The real party-in-interest is Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, CA 92008. Life Technologies, Inc. was the assignee of the entire right, title and interest in U.S. Application Serial No. 09/058,350. Life Technologies, Inc. has merged with Invitrogen Corporation, with Invitrogen Corporation being the surviving entity.

II. Related Appeals and Interferences (37 C.F.R. § 1.192(c)(2))

This application has not previously been before the Board of Patent Appeals and Interferences. Appellant's undersigned representatives are not aware of any related appeals and/or interferences within the meaning of 37 C.F.R. § 1.192(c)(2).

III. Status of the Claims (37 C.F.R. § 1.192(c)(3))

There are 32 claims in the application. There were 20 claims in the application as filed on April 10, 1998. Claim 1 was cancelled, claims 21 and 22 were added and claims 2-5, 7, 8, 10, 12, 13, and 19 were amended in the amendment filed June 3, 1999. Claim 12 was cancelled and claims 2, 3, 21 and 22 were amended in the amendment filed February 16, 2000. Claim 3 was amended in the amendment filed November 8, 2000. Claims 23-34 were added in the supplemental amendment on January 16, 2001. No further amendments to the claims were made in the Reply After Final filed on September 26, 2001.

Accordingly, the claims on appeal are 2-11 and 13-34. A copy of the claims on appeal can be found in the attached Appendix as required under 37 C.F.R. § 1.192(c)(9).

IV. Status of the Amendments (37 C.F.R. § 1.192(c)(4))

All amendments have been entered. No amendments to the claims were presented subsequent to the final rejection.

V. Summary of the Invention(37 C.F.R. § 1.192(c)(5))

A. Concise Statement of the Invention

In general, the invention relates to methods, extraction reagents and kits for isolating ribonucleic acid (RNA) from eukaryotic cells, such as plant or animal cells. ***(See page 1, lines 4-6)***. More specifically, the invention relates to an extraction reagent for RNA isolation comprising a phenol, a nonionic detergent and a phenol solubilizer (claim 21) or a chelator (claim 22). ***(See original claim 1.)*** Dependent claims further limit claims 21 and/or 22, by e.g. reciting narrower concentration ranges, by more specifically identifying components and/or by reciting additional components. ***(See page 4, line 15 through page 5, line 15).*** The invention also relates to methods (claims 13-20) that use the extraction reagents of the invention ***(See page 8, line 15-25).***

Previous reagents and methods co-precipitate DNA with insoluble cell debris ***(See page 1, lines 16-23)*** or require expensive RNase inhibitors and extensive sample handling to recover purified mRNA ***(See page 2, lines 13-25)***. Therefore, the invention is directed to improved methods and reagents for RNA isolation.

VI. Issues Presented for Review (37 C.F.R. 1.192(c)(6).

In the final Office Action mailed March 27, 2001 (Paper No. 12), the Examiner rejected claims 2-11 and 13-34 under 35 U.S.C. § 103 as allegedly being unpatentable over Sambrook *et al.,* (Molecular Cloning: A Laboratory Manual - hereinafter "Sambrook), in

combination with Chomczynski (U.S. Patent No. 5,346,994 - hereinafter "Chomczynski) and Perlman (U.S. Patent No. 5,098,603 - hereinafter "Perlman").

In the Advisory Action mailed October 31, 2001, it was indicated that Appellant's request for reconsideration did not place the application in condition for allowance for the reasons of record in the action mailed August 16, 1999 and March 27, 2001.

Accordingly, the issues presented for review are as follows:

A. Whether claims 21, 28, 29, and claims 2, 3 and 7 to the extent that they depend on claim 21, which are directed to an aqueous RNA extraction reagent, comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one phenol solubilizer at a concentration of 15-55% (vol/vol), are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

B. Whether claims 6, 22, 23 and 24 and claims 2, 3 and 7 to the extent that they depend on claim 22, which are directed to an aqueous RNA extraction reagent, comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one chelator at a concentration of 0.02-0.25 M, are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

C. Whether claims 4 and 5 to the extent that they depend on claim 21, which are directed to an aqueous RNA extraction reagent comprising octylphenoxypoly(oxyethylene) ethanol at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one phenol solubilizer at a concentration of 15-55% (vol/vol) are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing NP-40, in view of Chomczynski which describes an RNA,

DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

D. Whether claims 4 and 5 to the extent that they depend on claim 22, which are directed to an aqueous RNA extraction reagent, comprising octylphenoxy-poly(oxyethylene) ethanol at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one chelator at a concentration of 0.02-0.25 M are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

E. Whether claims 8 and 9, which are directed to an aqueous RNA extraction reagent, comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol), at least one chelator at a concentration of 0.02-0.25 M and at least on phenol solubilizer at a concentration of 15-55% (wgt/vol) are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

F. Whether claims 10 and 11 to the extent that they depend on claim 21, which are directed to an aqueous RNA extraction reagent comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol), at least one phenol solubilizer at a concentration of 15-55% (vol/vol) and at least one phenol stabilizer at a concentration of 0.05%-0.2% are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

G. Whether claims 10 and 11 to the extent that they depend on claim 22, which are directed to an aqueous RNA extraction reagent,

comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol), at least one chelator at a concentration of 0.02-0.25 M and at least one phenol stabilizer at a concentration of 0.05%-0.2% are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

H. Whether claims 25-27 and 31, which are directed to an aqueous RNA extraction reagent comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol), at least one phenol solubilizer at a concentration of 15-55% (vol/vol) and sodium citrate at a concentration of 0.02-0.25 M are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution but not citrate as a chelator?

I. Whether claims 30, 32 and 33, which are directed to an aqueous RNA extraction reagent comprising octylphenoxy poly(oxyethylene)ethanol at a concentration of about 0.5%, 8-hydroxyquinoline at a concentration of 0.05-0.2%, sodium citrate at a concentration of 0.02-0.25 M, at least one phenol at a concentration of 10-60% (wgt/vol), at least one phenol solubilizer at a concentration of 15-55% (vol/vol) and sodium citrate at a concentration of 0.02-0.25 M are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution but not citrate as a chelator?

J. Whether claim 34 which is directed to an aqueous RNA extraction reagent comprising 1) at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one phenol solubilizer at a concentration of 15-55% (vol/vol), or 2) comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least

one phenol at a concentration of 10-60% (wgt/vol) and at least one chelator at a concentration of 0.02-0.25 M, said reagent containing a salt of citric acid at a concentration of 0.05-0.2% is obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution not the use of a salt of citric acid?

K. Whether claims 13-20 to the extent that they depend on claim 21, which are directed to a method for providing cytoplasmic RNA from a cell sample comprising eukaryotic cells, comprising : (a) mixing said sample containing said cells with an RNA extraction reagent comprising a non-ionic detergent (0.1-1.0%), a phenol (10-60%) and a phenol solubilizer (15-55%) form a mixture; (b) adding a haloalkane to the mixture and mixing the resulting organic and aqueous phases; (c) separating the organic and aqueous phases; and (d) precipitating cytoplasmic RNA from the aqueous phase obtained in step (c) is obvious in view of Sambrook, Chomczynski and Perlman, none of which teach the above steps for providing cytoplasmic RNA?

L. Whether claims 13-20 to the extent that they depend on claim 22 which are directed to a method for providing cytoplasmic RNA from a cell sample comprising eukaryotic cells, comprising: (a) mixing said sample containing said cells with an RNA extraction reagent comprising a non-ionic detergent (0.1-1.0%), a phenol (10-60%) and a chelator (0.02-0.25 M) to form a mixture; (b) adding a haloalkane to the mixture and mixing the resulting organic and aqueous phases; (c) separating the organic and aqueous phases; and (d) precipitating cytoplasmic RNA from the aqueous phase obtained in step (c) is obvious in view of Sambrook, Chomczynski and Perlman, none of which teach the above steps for providing cytoplasmic RNA?

VI. Grouping of the Claims.

All of the claims on appeal do ***not*** stand or fall together. The claims have been separately grouped because the claimed RNA extraction reagents have different patentably distinct features.

A. Claims 21, 28, and 29 and claims 2, 3 and 7, to the extent that they depend on claim 21, stand together.

B. Claims 6, 22, 23, and 24 and claims 2, 3 and 7, to the extent that they depend on claim 22, stand together.

C. Claims 4 and 5, to the extent that they depend on claim 21, stand together.

D. Claims 4 and 5, to the extent that they depend on claim 22, stand together.

E. Claims 8 and 9 stand together.

F. Claims 10 and 11, to the extent that they depend on claim 21, stand together.

G. Claims 10 and 11, to the extent that they depend on claim 22, stand together.

H. Claims 25-27 and 31 stand together.

I. Claims 30, 32 and 33 stand together.

J. Claim 34.

I. Claims 13-20, to the extent that they depend on claim 21, stand together.

J. Claims 13-20, to the extent that they depend on claim 22, stand together.

VII. Arguments

A. ***Issue A (claims 21, 28, and 29 and claims 2, 3, and 7, to the extent that they depend on claim 21.)*** Whether the claimed RNA extraction reagent comprising at least one nonionic detergent (0.1-1.0%), at least one phenol (10-60%) and at least one phenol solubilizer (15-55%), is *prima facie* obvious in view of Sambrook, which describes an RNA extraction buffer lacking both phenol and phenol solubilizer but containing a non-ionic detergent, Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which describes a chelated phenol solution?

1. The Examiner' Obviousness Rejection

A Final Office Action was mailed March 27, 2001, [Paper 12] that repeated an earlier rejection of claims 2-11 and 13-22 under 35 U.S.C. § 103 over Sambrook, Chomczynski and Perlman. In this rejection the Examiner described the claims as two groups (the first group being claims 2-11 and the second group being claims 13-34). The rejection ignored the different limitations in the dependent claims.

Appellant filed a lengthy and detailed Reply After Final (20 pages) on September 26, 2001. In the reply, Appellant again indicated to the Examiner that there was confusion over the manner in which the claims were being examined and that many of the claims ***contained additional limitations that required consideration.*** Further, it was brought to the Examiner's attention that claims to a reagent were being rejected based on a perceived interpretation of art describing "methods" of isolating or purifying nucleic acids.

Following the Reply After Final, Appellant received an Advisory Action mailed on October 31, 2001, [Paper 22] that failed to address any of the arguments raised in Appellant's detailed twenty-page Reply After Final. The only explanation in the Advisory Action for maintaining the rejection was for "reasons of record set forth in the office actions mailed 8/16/99 and 3/27/01."

2. The Obviousness Rejection is in Error and Must be Reversed

Appellant respectfully submits that the Examiner's rejection is in error because the Examiner has failed to establish the requisite suggestion or motivation to combine the cited

art. The rejection is further in error at least because inappropriate hindsight analysis has been used in an attempt to selectively combine the art to arrive at the claimed invention. Since the Examiner has failed to establish a *prima facie* case of obviousness, the rejection of claims 21, 28, 29 and claims 2, 3, and 7, to the extent that they depend on claim 21, must be reversed.

a. There is no motivation or suggestion to combine Sambrook, Chomczynski and Perlman to obtain the claimed invention

As one requirement to establish a *prima facie* case of obviousness of claims 21, 28, 29 and claims 2, 3 and 7 to the extent they depend on claim 21, the Examiner must show that there was a suggestion or motivation to combine the cited art, i.e. Sambrook, Chomczynski and Perlman to obtain an RNA extraction reagent comprising at least one non-ionic detergent, at least one phenol and at least one phenol solubilizer, each of which being present in a concentration range of 0.1-1.0%, 10-60% and 15-55%, respectively. Thus, the Examiner must establish that the prior art suggested not only the combination of specific individual components, but also the concentration range of each of the components in the complete reagent. The Examiner has provided no suggestion or motivation whatsoever for combining Sambrook with Chomczynski and Perlman to obtain Appellant's claimed RNA extraction reagent comprising a non-ionic detergent, a phenol and a phenol solubilizer in the recited concentration ranges. In the absence of such a suggestion or motivation, there can be no *prima facie* case of obviousness.

i. There is no motivation to combine the cited art to obtain an RNA extraction reagent comprising a non-ionic detergent, a phenol and a phenol solubilizer.

The § 103 rejection using the combination of Sambrook, Chomczynski and Perlman was first raised in the Office Action of May 9, 2000. This combination fails to suggest an RNA extraction reagent comprising comprising at least one non-ionic detergent, at least one phenol and at least one phenol solubilizer, each of which being present in a concentration range of 0.1-1.0%, 10-60% and 15-55%, respectively.

Sambrook (Page 7.6), the primary reference, refers to an "RNA extraction buffer" having 0.5% NP40, but containing ***neither*** a phenol or a phenol solubilizer. Sambrook also discusses a method for RNA isolation. According to the method of Sambrook at page 7.7 under the heading "Lysis of cells . . . ," one follows a three step procedure. First, one combines an equal volume of the RNA extraction buffer containing NP-40 with an equal volume of a "digestion buffer." A proteinase K solution is next added and one incubates this for 30 minutes. This is then followed by addition of an equal volume of phenol:chloroform. Even assuming, *arguendo*, that the phenol:chloroform solution referred to at 7.7 of Sambrook[1], was the same as that described in Appendix E.3, i.e. it contained phenol:chloroform:isoamyl alcohol (25:24:1 = 2% isoamyl alcohol), this solution is then diluted 1:1 and the final concentration of isoamyl alcohol (described by Sambrook as a foam reducing agent but which however, may also be a phenol solubilizer) would be 1%, i.e. ***less than 7%*** of the minimum or ***less than 2%*** of the maximum phenol solubilizer concentration required for the concentration range of claim 21 (15-55% phenol solubilizer). Thus,

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[1]This, however, is by no means clear from the direction given in Sambrook at 7.7.

Sambrook clearly fails to suggest the complete RNA extraction reagent having the appropriate concentrations of each component recited in claim 21.

Chomczynski, a secondary reference, discusses use of phenol at 30-50% and glycerol (a phenol solubilizer) in the range of about 3-10%[2], ***not*** 15-55% as required for a phenol solubilizer in the reagent of claim 21. Thus, the highest end of the concentration range (10%) of Chomczynski for a phenol solubilizer is only 67% (i.e. 10%/15%) of the lower range of the phenol solubilizer concentration (15%) in claim 21. Moreover, ***nowhere***, does Chomczynski or the other cited art teach or suggest that a nonionic detergent may be added to this mixture.

Perlman, a secondary reference discusses a chelated phenol solution and adds nothing to remedy the deficits in Sambrook or Chomczynski.

DeBonville (U.S. Patent No. 4,833,239), which was ***not*** part of the formal rejection but was referred to in the rejection, does nothing to shed light on why Sambrook, Chomczynski and Perlman should be combined. Further, nothing provided in DeBonville would render obvious the individual component concentrations in the invention of claim 21.

At best, DeBonville's ***DNA isolation reagent*** of Example 1 (Column 4, lines 10-23) contains 0.2% isoamyl alcohol (a solubilizer) in combination with 9.9% phenol. In Example 2 (Column 4, lines 26-47), the solution of Example 1 containing 9.9% phenol is then added to a second solution resulting in an approximate 1:3 dilution. This results in a complete ***DNA isolation reagent***, not an RNA extraction reagent, having a final phenol concentration of approximately 3.3%, approximately 0.066% phenol solubilizer with a detergent concentration

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[2]In the Office Action mailed May 9, 2000 [Paper 11] (page 3, third paragraph), the Examiner ***incorrectly*** stated that Chomczynski teaches use of a solubilizer at 3-15% in claim 8. Claim 8 of Chomczynski actually refers to a solubilizer at a concentration of 3-10%.

of 0.45% SDS. SDS, however, is ***not*** a non-ionic detergent. Therefore, even if DeBonville was formally part of the rejection it would still fail to render obvious the concentration of a phenol and a phenol solubilizer as used in the claimed invention. Further, the reagent of DeBonville does not even include a non-ionic detergent, but does include an RNase.

Therefore, the reagent of DeBonville is deficient for multiple reasons: a) it is a DNA isolation reagent ***not*** an RNA extraction reagent, b) it does ***not*** suggest a phenol concentration of 10-60%, c) it does ***not*** suggest a phenol solubilizer concentration of 15-55%, d) it does ***not*** contain a non-ionic detergent and d) it would degrade RNA which is clearly an undesirable characteristic for an RNA extraction reagent. Thus, DeBonville does nothing to further explain away the deficits in the combination of Sambrook, Chomczynski and Perlman.

The Examiner's reasoning for combining Sambrook with Chomczynski and Perlman was never explicitly stated in ***any*** of the Office Actions and the rationale for combining the cited art is at best, based on no more than a conclusory argument. In the Office Action of May 9, 2000 [Paper 11], the Examiner stated that "[i]t would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to combine the selected reagents in kit form to extract RNA." (Office Action of May 9, 2000 at page 4, lines 10-12). The issue is whether there is a motivation or suggestion to combine the cited art to obtain an RNA extraction reagent comprising a non-ionic detergent, a phenol and a phenol solubilizer. This motivation or suggestion has not been established.

It is respectfully submitted that the Examiner has provided no suggestion or motivation to combine the cited art and has done no more than indiscriminately combine that art, absent any motivation to do so. This is not permitted. *See Micro Chemical v. Great*

Plains Chemical Co., 41 U.S.P.Q.2d 1238, 1244 (Fed. Cir. 1997) ("A determination of obviousness must involve more than indiscriminately combining prior art; ***a motivation or suggestion to combine must exist.***") (Emphasis added).

The alleged rationale for combining the art was articulated in somewhat more detail in the Office Action of May 9, 2000 as being that one of ordinary skill in the art is "motivated to combine the teaching of Perlman regarding the inclusion of a chelator with phenolic solutions with the nucleic acid extraction reagents of DeBonville[3] (U.S. Patent No. 4,833,239) and Chomczynski to prevent oxidation of phenol by divalent metal ions." (Paper No. 11 at page 4, lines 20-24). This same alleged motivation was repeated in the Office Action of March 27, 2001 [Paper No. 12] at page 4, lines 11-16, without further elaboration concerning the combination of all three pieces of cited art (i.e. Sambrook with Chomczynski and Perlman). No further motivation, has been provided for adding Perlman to the combination of art rejecting claim 21[4].

The issue is whether one is motivated to selectively combine Sambrook, Chomczynski and Perlman to obtain an RNA extraction reagent comprising a non-ionic detergent, a phenol and a phenol solubilizer, not whether one wants "to prevent oxidation of phenol." ***Nowhere*** is there a suggestion to combine the art to obtain a reagent having all the same components as the reagent of claim 21. Multiple reagents that may individually contain

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[3]DeBonville is not formally part of the rejection, however, Appellants will briefly discuss it in the next section.

[4]Appellant notes, however, that in Paper No. 12 at the top of page 4, the Examiner did state ". . . the prior art has set forth the motivation for the use of the reagent cited supra in extraction reagents of RNA," without further elaboration as to what that motivation would be. Again, this is no more than a conclusory argument. No motivation to combine the art has been provided.

components found in Appellant's reagent do not render obvious the invention. Furthermore, use of reagents in the cited art result in multi-step extractions while Appellant's reagent may be used in a single-step extraction.

Perlman which discusses a chelated phenol solution is ***completely irrelevant*** to a combination of art that would result in an RNA extraction reagent comprising a non-ionic detergent, a phenol and a phenol solubilizer, because such a reagent does not require a chelator. Perlman discusses only the use of a chelator with a phenol solution. The additional argument to combine Perlman with Chomczynski (i.e to obtain a phenol solution with a chelator) fails to provide any basis for combining a non-ionic detergent, a phenol and a phenol solubilizer to obtain Appellant's invention of claims 21, 28, 29 and claims 2, 3, and 7 to the extent they depend on claim 21.

Sambrook, the primary reference, discusses an RNA extraction buffer at page 7.6 that contains ***neither*** a phenol ***nor*** a phenol solubilizer. Neither Appendix B5 nor E3 of Sambrook cited by the Examiner [Paper No. 12] remedies this deficit, because they discuss either phenol-chloroform extraction of proteins or a phenol preparation but ***not*** a reagent containing the components of claim 21. The phenol-chloroform extraction is an extraction method used to separate components between two phases- an organic phase and an aqueous phase. This chemical extraction is not at all relevant to the extraction of RNA from cells. Sambrook's reagent at page 7.6, however, does contain Nonidet P-40 (NP40), i.e a non-ionic detergent.

At page 7.7, Sambrook discusses a separate and distinct solution used after lysis of cells for removing proteins (***not*** an RNA extraction reagent) having phenol and chloroform. This ***separate*** reagent (i.e. the phenol:chloroform), however, does not contain NP40. Thus,

Sambrook discusses two separate and distinct reagents, neither of which individually would contain all the components at the concentrations of the reagent of claim 21.

Chomczynski, a secondary reference, discusses use of phenol and glycerol (a phenol solubilizer). Chomczynski fails to suggest how to modify Sambrook to arrive at the claimed invention. Further, ***nowhere*** does Chomczynski teach or suggest adding a non-ionic detergent such that one may obtain an RNA extraction reagent containing a phenol, phenol solubilizer and a non-ionic detergent. To do so would interfere with the phase separation. Neither Sambrook nor Perlman suggest addition of a non-ionic detergent to the reagent of Chomczynski.

As noted above, Perlman discusses no more than stabilizing phenol solutions using a chelator. There is no suggestion in the cited art to add a phenol solubilizer and a non-ionic detergent to Perlman to obtain the claimed invention, nor is there anything in Perlman to suggest combining Sambrook and Chomczynski. Therefore, Perlman adds nothing to remedy the deficits in Sambrook or Chomczynski.

In the Advisory Action [Paper No. 22], one basis for rejecting claims 2-11 and 13-34 was for the reasons of record in the Office Action of August 16, 1999 [Paper No. 9]. That action, however, failed to provide any motivation to combine the cited art and in fact supported Appellant's position that there is ***no*** basis to combine the cited art. In Paper 9, a previous rejection of claims 2-20 under 35 U.S.C. § 103 in view of Sambrook and Chomczynski was "withdrawn in view of applicant's arguments that the rejection ***lacks sufficient motivation*** for combining the references in such a manner as to arrive at applicant's invention." (Emphasis added) [Paper No. 9 at page 2]. Thus, it was acknowledged during prosecution that there was no motivation to combine at least Chomczynski and Sambrook.

Perlman was not a part of that earlier rejection, but nothing further has been added to the rejection to provide a motivation for combining Perlman with Sambrook and Chomczynski.

None of the cited art discusses or suggests an RNA extraction reagent having the three components recited in claim 21. Further, none of the cited art suggests combining separate individual components in order to obtain an RNA extraction reagent having a non-ionic detergent, a phenol and a phenol solubilizer as in claim 21. Therefore, for all of the above reasons, the Examiner has failed to provide a suggestion or motivation to combine the cited art to obtain the RNA extraction reagent of claims 21, 28, 29 and claims 2, 3, and 7, to the extent that they depend on claim 21. Appellant respectfully requests that the rejection of claims 21, 28, 29 and claims 2, 3, and 7, to the extent that they depend on claim 21, be reversed.

ii. DeBonville referred to by the Examiner (but not part of the formal rejection) fails to provide any basis for combining Sambrook, Chomczynski and Perlman.

The Examiner repeatedly referred to DeBonville in discussing the rejections, however, it was never made part of the formal rejection. For the sake of completeness, however, DeBonville is now further discussed. DeBonville is directed to methods of isolating DNA, ***not*** RNA. There is nothing in DeBonville that would provide a motivation for one skilled in the art to combine Sambrook, Chomczynski and Perlman.

As noted by DeBonville, ***a preferred DNA isolation method employs RNase A which degrades RNA*** (see col. 3, lines 62-64). Clearly, one isolating RNA would not find a motivation to combine a method of DNA isolation that includes an enzyme for degrading and digesting RNA which would therefore fail to result in an intact RNA preparation. Inclusion of RNase A in an RNA extraction reagent would clearly defeat the purpose using such a

reagent. Therefore, DeBonville does nothing to provide a motivation for combining Sambrook, Chomczynski and Perlman.

iii. Inappropriate Hindsight Analysis Has Been Used in an Attempt to Reconstruct the Claimed Invention

The Examiner has used inappropriate hindsight in an attempt to combine Sambrook, Chomczynski and Perlman by picking and choosing among isolated disclosures within the cited art and using Appellant's disclosure as a template for obtaining the invention. Repeatedly, the Examiner has made this error by failing to point to any art or combination of art that suggests a complete RNA extraction reagent comprising a non-ionic detergent (0.1-1.0%), a phenol (10-60%) and a phenol solubilizer (15-55%). Rather, the Examiner has attempted to reconstruct the reagent, by combining components found in distinct steps from disparate methods or reagents based on the template for the reagent provided in Appellant's application.

It is error for the Examiner to use Appellant's application as a template upon which to construct the claimed invention from the cited art. *See Ecolochem Inc. v. Southern California Edison*, 56 U.S.P.Q.2d 1065, 1072 (Fed. Cir. 2000) *citing In re Fine*, 5 U.S.P.Q.2d, 1780, 1783 (Fed. Cir. 1998) (reiterating the position and stating that: "We cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.").

In this regard, the Examiner has reconstructed the claimed invention by picking and choosing disparate components from the cited art using knowledge only obtained from the specification (which otherwise would not have been available). Specifically, the Examiner has picked a non-ionic detergent from the reagent of Sambrook and has picked phenol and

a phenol solubilizer from Chomczynski or a different reagent of Sambrook and then combined the two, while ignoring 1) the remaining components of each reagent 2) the individual component concentration and 3) the purposes for which they are to be used. This picking and choosing was done without any guidance found in the art or elsewhere or concern for what the art teaches as a whole. Therefore, this is an inappropriate basis for making an obviousness rejection.

McLaughlin was cited in the Office Action of March 27, 2001 at page 3 to support the use of hindsight by the Examiner. In the Reply After Final of September 26, 2001 under 37 C.F.R. §1.116 (beginning at the last line of page 9), Appellant argued that *McLaughlin* failed to support the rejection because 1) Appellant's reagents had not previously been used in combination for RNA extraction, 2) *McLaughlin* cautioned against using *only* knowledge gleaned from Appellant's disclosure and 3) the combination of the art's disclosure must be taken as a whole.

At best, the Examiner has done no more than use Appellant's specification and claims as the template for combining the cited art to obtain the claimed invention. This is clearly distinct from a motivation to combine references (*Uniroyal Inc. v. Rudkin-Wiley Corp.*, 837 F.2d 1044 (Fed. Cir. 1988). *Uniroyal* stated that:

> [T]here must be some reason for the combination [of prior art references] other than the hindsight gleaned from the invention itself. Something in the prior art as a whole must suggest the desirability, and thus the obviousness, of making the combination. It is impermissible to use the claims as a frame and the prior art references as a mosaic to piece together a facsimile of the claimed invention.

As such, because hindsight was improperly used, and because there was no suggestion or motivation to combine the art as was done, the rejection of claims 21, 28, and 29, and claims 2, 3, and 7, to the extent that they depend on claim 21, should be reversed.

iv. There was no suggestion to combine the cited art to obtained the claimed invention because none of the art teaches or suggests the components of the reagent in the required concentration ranges.

While individual or isolated elements of the claimed invention might have been mentioned in the cited art, the mere identification of individual elements is not enough to establish a *prima facie* case of obviousness. ***Nowhere*** in the cited art is there a teaching or suggestion to make the RNA extraction reagent having all the limitations found in claims 21, 28, 29 and claims 2, 3, and 7, to the extent that they depend on claim 21. None of the cited art, either individually or in combination, suggest an RNA extraction reagent comprising a non-ionic detergent at a concentration of 0.1-1.0%, a phenol at a concentration of 10-60% and a phenol solubilizer at 15-55%.

In fact, ***nowhere*** in any of the cited art is there a teaching or suggestion to make ***any reagent whatsoever*** having a phenol solubilizer at a concentration of 15-55% (vol/vol). Thus, there is no motivation to combine the cited art to obtain the claimed invention because specific component concentrations are ***not*** taught anyplace in the art.

In this regard, the Examiner argued in the Office Action of March 27, 2001 [Paper No. 12] that:

> Although the identical concentration ranges ***are not disclosed*** in the references cited supra, proportions of ingredients, to impart patentability to an ***otherwise obvious*** chemical composition, must produce more than a mere difference in degree in the properties of the composition. The proportions must be critical, i.e. they must produce a difference in kind rather than degree, which is not seen in the instant application.

(Emphasis added).

This fails to provide an appropriate basis for rejecting Appellant's claimed invention under 35 U.S.C. § 103 because ***nowhere*** has the Examiner actually shown that a reagent having all of the ***same components*** at even approximately the ***same concentrations*** was "otherwise present" in the art. Furthermore, differences between concentrations of the components in Appellant's invention and the concentrations of individual components in the cited art are not minor. Even assuming *arguendo*, that the differences between the component concentration in the art and that of the claimed invention were minor, the art must still be evaluated in terms of the invention as a whole. This has not been done. In *Northern Telecom, Inc. v. Datapoint Corp.*, 15 U.S.P.Q.2d 1321, 1324 (Fed. Cir. 1990), the Federal Circuit referred to *Fromson v. Advance Offset Plate, Inc.* (225 U.S.P.Q. 26, 31 (Fed. Cir. 1985) and stated that:

> 'prior art must suggest to one of ordinary skill in the art the desirability of the claimed combination.' Whether the changes from the prior art are 'minor', as Datapoint argues, the changes must be evaluated in terms of the whole invention, including whether the prior art provides any teaching or suggestion to one of ordinary skill in the art to make the changes that would produce the patentee's method and device. [citation omitted]

Additionally, the decisions upon which the Examiner's argument concerning "criticality" of the components is most likely based are inapposite to the basis for the rejection. (*See e.g.*, *In re Woodruff*, 16 U.S.P.Q.2d 1934 (Fed. Cir. 1990), *Merck & Co. v. Biocraft Labs. Inc,* 10 U.S.P.Q.2d 1843 (Fed. Cir. 1989), *In re Aller*, 105 U.S.P.Q. 233 (C.C.P.A. 1955)). In each of these cases, the complete composition or method was already known from a ***single*** piece of art.

Contrary to the above, Appellant's reagent was not previously known and therefore, before even addressing the criticality issue, the Examiner must properly attempt to combine

Sambrook, Chomczynski and Perlman to obtain a reagent having all the components of Appellant's reagent, in an attempt to render the claimed invention obvious. As argued above, there is no motivation to combine the cited art. Therefore, the criticality argument does not mitigate the fact that the art fails to suggest the concentrations as set forth in the claims.

Clearly, when one considers the numerous possible concentration ranges of individual components used in different methods of the cited art, it cannot be said that one of skill in the art would be motivated to combine Sambrook, Chomczynski and Perlman to obtain ***the specific combination of components and the specific concentrations of those components as required in Appellant's claimed reagent.***

The Examiner has done nothing more than modify cited art merely because "one could" make such a modification. This is clearly an incorrect approach to obtaining the claimed invention. The mere fact that a reference teaching could conceivably be combined with another reference teaching or otherwise modified does not render the resultant combination or modification obvious unless the prior art also suggests the desirability of that specific combination or modification. (*See In re Mills*, 16 U.S.P.Q.2d 1430 (Fed. Cir. 1990)). Moreover, an assertion that it would have been well within the ordinary skill in the art at the time the invention was made to modify the cited art to achieve the claimed invention merely because the references relied upon teach that all aspects of the claimed invention were *individually* known in the art is not sufficient to establish a *prima facie* case of obviousness without some *objective* reason to combine the specific teachings of the references. (MPEP § 2143.01.)

Nowhere is there any teaching or suggestion of any extraction reagent whatsoever, having a phenol solubilizer at a concentration of 15-55% (vol/vol), let alone a single RNA

extraction reagent having a phenol (10-60%), a phenol solubilizer (15-55%) and a non-ionic detergent (0.1-1.0%). Additionally, there is no guidance as to how or why one would modify the cited art in order to obtain such a reagent. As such, there is no motivation to combine the cited art in an attempt to obtain the claimed invention.

Therefore, Appellant respectfully requests that the rejection of claims 21, 28, and 29, and claims 2, 3, and 7, to the extent that they depend of claim 21, be reversed.

B. ***Issue B (claims 6, 22, 23, and 24, and claims 2, 3, and 7, to the extent that they depend on claim 22)*** Whether an aqueous RNA extraction reagent, comprising at least one nonionic detergent at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60% and at least one chelator at a concentration of 0.02-0.25 M is obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 6, 22, 23, and 24, and claims 2, 3, and 7, to the extent they depend on claim 22. Therefore, the comments in section ***VII.A.1*** are incorporated herein by reference. Claim 22 is directed to an aqueous RNA extraction reagent, comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one chelator at a concentration of 0.02-0.25 M. This reagent is clearly distinct, from the reagent of claim 21 because rather than containing a phenol solubilizer, the reagent of claim 22 contains a chelator at 0.02-0.25 M.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in section ***VII.A.2*** are incorporated herein by reference because the Examiner has failed to distinguish these claims from the previous group of claims despite different limitations. Appellant further submits that the Examiner has provided no teaching, suggestion or reason why one of ordinary skill in the art would prepare an RNA extraction reagent comprising a chelator at a concentration of 0.02-0.25 as required by claims 6, 22, 23 and 24.

Appellant submits that the Examiner has failed to establish a *prima facie* case of obviousness for claims 6, 22, 23, and 24, and claims 2, 3, and 7, to the extent they depend on claim 22. An important element of the reagent, the presence of a chelator at 0.02-0.25 M is absent from the cited art. The rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure.

In order to render claim 22 obvious over the cited art, the Examiner must provide a motivation in the prior art to combine the art in order to obtain a reagent containing a non-ionic detergent, phenol and a chelator. This has not been done.

There was no motivation or suggestion to combine Perlman with Sambrook and Chomczynski. This argument was made in detail in ***VII.A.2*** (which is incorporated herein by reference) as it related to an RNA extraction reagent comprising at least one non-ionic detergent, at least one phenol and at least one phenol solubilizer. The arguments are equally applicable to the Examiner's attempt to combine the cited art to obtain an RNA extraction

reagent comprising at least one non-ionic detergent, at least one phenol, and at least one ***chelator***.

The Examiner has argued that one of ordinary skill in the art is "motivated to combine the teaching of Perlman regarding the inclusion of a chelator with phenolic solutions" with the nucleic acid extraction reagents of Chomczynski and Sambrook "to prevent oxidation of phenol by divalent metal ions." (Paper No. 11 at page 4, lines 20-24). Merely wanting to prevent "oxidation of phenol" is not the same as a motivation to combine the cited art to obtain the an RNA extraction reagent comprising at least one non-ionic detergent, at least one phenol and at least one chelator.

The difference in the concentration of EDTA (a chelator) in Perlman relative to that in the invention is *not* minor. Referring to Perlman at page 6, second full paragraph of the reply filed November 8, 2000 Appellant previously stated:

> Therefore, at best, the recitation pointed to by the Examiner teaches adding a *specific* concentration of EDTA, i.e. *0.1 to 10 mM* (preferably 1 mM) to a phenol solution, not an RNA isolation reagent. Contrary to this, claim 22 recites "at least one chelator at a concentration of 0.02-0.25 M" (i.e. 20 mM-250 mM) in a composition with additional components.

(Emphasis in original).

Even assuming, *arguendo*, that Perlman was appropriately combined this would still fail to suggest an RNA extraction reagent having 20-250 mM chelator. The higher end of Perlman's EDTA concentration range (10 mM) is ***only*** half of Appellant's lowest chelator concentration (0.02 M = 20 mM). Moreover, the suggested preferred EDTA concentration (i.e. 1 mM) in Perlman is only 5% of the lower end of Appellant's lower EDTA concentration range (20 mM) and less than 0.5% of the high end (0.25 M = 250 mM) of Appellant's concentration range. There is no reason whatsoever to assume that the concentration of at

least the chelator in Appellant's extraction reagent of claim 22 would have been obvious in view of Perlman, Sambrook and Chomczynski.

The Examiner further attempts to justify the use of Perlman in the combination of art because it "adequately bridges the nexus between the differences in the prior art and the invention as claimed." "Bridging a nexus" is not the same as "providing a motivation" to combine the art and is not a sufficient basis for combining the art. Thus there is no suggestion or motivation to combine Perlman with Chomczynski and Sambrook.

DeBonville also referred to by the Examiner, although not part of the formal rejection, uses a chelator (8-hydroxyquinoline) in a DNA isolation reagent (Column 4, lines 10-47). The final concentration of the chelator in DeBonville's complete reagent described at column 4, lines 45-46 of that patent is no more than approximately 0.46 mM. This is more than an order of magnitude less than the minimum of 20 mM found in Claim 22. The additional description of 10 mM EDTA (line 36) still fails to provide a motivation or suggestion to combine Sambrook with Chomczynski and Perlman because the EDTA is diluted approximately 5-fold in the complete solution resulting in a final concentration of approximately 2 mM. This is still approximately 10-fold less than the minimum concentration recited for a chelator in claim 22.

Therefore, for at least all the above reasons and also for all of the reasons previously set forth, Appellant respectfully requests that the rejection of claims 6, 22, 23, and 24 and claims 2,3, and 7, to the extent they depend on claim 22, be reversed.

C. Issue C (claims 4 and 5 to the extent that they depend on claim 21)

Whether an aqueous RNA extraction reagent comprising octylphenoxypoly (oxyethylene) ethanol at a concentration of 0.1%-1.0%, at least one phenol at a concentration of 10-60% and at least one phenol solubilizer at a concentration of 15-

55% is obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent (NP-40), in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 4 and 5 (to the extent they depend on claim 21) which are directed to an aqueous RNA extraction reagent, comprising octylphenoxypoly(oxyethylene) ethanol at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60% and at least one phenol solubilizer at a concentration of 15-55%. Therefore, the comments in section ***VII.A.1*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in section ***VII.A.2.*** are also incorporated herein by reference. Appellant further submits that the Examiner has provided no guidance concerning why one would combine the cited art to obtain an RNA extraction reagent having the non-ionic detergent octylphenoxypoly(oxyethylene) ethanol at a concentration of 0.1-1.0% (claim 4) or "about 0.5%" (claim 5), with a phenol and phenol solubilizer. Further, the Examiner has failed to point to anything in the art that would even suggest the specific non-ionic detergent used in theRNA extraction reagent of claims 4 and 5.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claims 4 and 5 to the extent they depend on claim 21. An important element of the invention, octylphenoxypoly(oxyethylene) ethanol at a concentration of 0.1-1.0%, is nowhere to be found in the cited art. Nowhere in any of the cited art has the Examiner

pointed to the use of octylphenoxypoly(oxyethylene) ethanol at any concentration whatsoever in an RNA extraction reagent.

The rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure. Where else could it have been found? In view of these additional remarks above, Appellant submits that the rejection of claims 4 and 5 to the extent that they depend on claim 21 must be reversed.

D. Issue D (claims 4 and 5 to the extent that they depend on claim 22)

Whether an aqueous RNA extraction reagent comprising octylphenoxypoly (oxyethylene) ethanol at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60% and at least one chelator at a concentration of 0.02-0.25 M is obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent (NP-40), in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 4 and 5 (to the extent they depend on claim 22) which are directed to an aqueous RNA extraction reagent, comprising octylphenoxypoly(oxyethylene) ethanol at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60% and at least one chelator at a concentration of 0.02-0.25 M. Therefore, the comments in sections ***VII.A.1*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in sections ***VII.A.2 and VII.B.2.*** are also incorporated herein

by reference. Appellant further submits that the Examiner has provided no teaching, suggestion or reason why one of ordinary skill in the art would combine the cited art to obtain an RNA extraction reagent having the non-ionic detergent octylphenoxypoly(oxyethylene) ethanol at a concentration of 0.1-1.0 (claim 5) or "about 0.5%" (claim 5), with at least one phenol and at least one chelator.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claims 4 and 5 to the extent they depend on claim 22. An important element of the invention, octylphenoxypoly(oxyethylene) ethanol at a concentration of 0.1-1.0%, is nowhere to be found in the cited art.

The rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure. Where else could it have been found? In view of the remarks above, Appellant submits that the rejection of claims 4 and 5 to the extent that they depend on claim 22 must be reversed.

E. ***Issue E (claims 8 and 9)*** Whether an aqueous RNA extraction reagent, comprising at least one nonionic detergent (0.1-1.0%), at least one phenol (10-60%), at least one chelator (0.02-0.25 M) and at least one phenol solubilizer (15-55%) is obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 8 and 9 which are directed to an aqueous RNA extraction reagent comprising at least non-ionic detergent at a concentration

of 0.1-1.0%, at least one phenol at a concentration of 10-60%, at least one chelator at a concentration of 0.02-0.25 M and at least one phenol solubilizer at a concentration of 15-55% ed herein by reference. Therefore, the comments in sections ***VII.A.1*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in sections ***VII.A.2 and VII.B.2*** are incorporated herein by reference. Appellant further submits that the Examiner has provided no teaching, suggestion or reason why one of ordinary skill in the art would combine the cited art to obtain an RNA extraction reagent comprising a non-ionic detergent at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60%, at least one chelator at a concentration of 0.02-0.25 M and ***further comprising*** at least one phenol solubilizer at a concentration of 15-55%.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claims 8 and 9. An important element of the invention is the addition of at least one phenol solubilizer to the RNA extraction reagent comprising at least one non-ionic detergent, at least one phenol and at least one chelator. Nowhere has the Examiner provided a suggestion or motivation to combine Sambrook with Chomczynski and Perlman to obtain the RNA extraction reagent of claims 8 and 9.

The rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure. Where else could the appropriate guidance have been found? In view of the remarks above, Appellant submits that the rejection of claims 8 and 9 must be reversed.

F. ***Issue F (claims 10 and 11 to the extent they depend on claim 21)*** Whether an aqueous RNA extraction reagent comprising at least one nonionic detergent (0.1-1.0%), at least one phenol (10-60%), at least one phenol solubilizer (15-55%) and at least one phenol stabilizer (0.05%-0.2%) is obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 10 and 11 (to the extent they depend on claim 21) which are directed to an aqueous RNA extraction reagent, comprising at least one non-ionic detergent at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60%, at least one phenol solubilizer at a concentration of 15-55% and at least one phenol stabilizer at a concentration of 0.05%-0.2%. Therefore, the comments in section ***VII.1.A*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in section ***VII.A.2.*** are incorporated herein by reference. Appellant further submits that the Examiner has provided no teaching, suggestion or reason why one of ordinary skill in the art would combine Sambrook, Chomczynski and Perlman to obtain an aqueous RNA extraction reagent, comprising at least one non-ionic detergent at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60%, at least one

phenol solubilizer at a concentration of 15-55% and at least one phenol stabilizer at a concentration of 0.05%-0.2%.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claims 10 and 11 to the extent they depend on claim 21. An important element of the invention as recited in claim 10 is the further addition of a phenol stabilizer at a concentration of 0.05%-0.2% in the complete extraction reagent. There is no suggestion to combine that cited art to obtain a reagent having the specific combination of components.

The Examiner did point to use of 8-hydroxyquinoline as a stabilizer in DeBonville [Paper 11, page 3, third paragraph], a member of the Markush group of claim 11. However, as pointed out earlier DeBonville is not part of the formal rejection. Further, the final concentration of the 8-hydroxyquinoline in DeBonville (approximately 0.016% - *See* column 4, lines 10-47) is about 3 times less than the minimum of 0.05% required for the reagents of claims 10 and 11.

The only manner by which the Examiner would have been able to obtain the invention of claims 10 and 11 to the extent they depend on claim 21 would have been to use Appellant's application to provide a template for obtaining the claimed reagent. The cited references simply do not get one there. Therefore, the rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure. In view of the remarks above, Appellant submits that the rejection of claims 10 and 11 to the extent that they depend on claim 21 must be reversed.

G. Issue G (claims 10 and 11 to the extent they depend on claim 22)

Whether an aqueous RNA extraction reagent comprising at least one nonionic detergent (0.1-1.0%), at least one phenol (10-60%), at least one chelator (0.02-0.25 M) and at least one phenol stabilizer (0.05%-0.2%) is obvious in

view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution?

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 10 and 11 (to the extent they depend on claim 22) which are directed to an aqueous RNA extraction reagent, comprising at least one non-ionic detergent at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60%, at least one chelator at a concentration of 0.02-0.25 M and at least one phenol stabilizer at a concentration of 0.05-0.2%. Therefore, the comments in section ***VII.A.1*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in sections ***VII.A.2., VII.B.2 and VII.G.2*** are incorporated herein by reference. Appellant further submits that the Examiner has provided no teaching, suggestion or reason why one of ordinary skill in the art would combine Sambrook, Chomczynski and Perlman to obtain an aqueous RNA extraction reagent, comprising at least one non-ionic detergent at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60%, at least one chelator at a concentration of 0.02-0.25 M and at least one phenol stabilizer at a concentration of 0.05-0.2%.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claims 10 and 11, to the extent they depend on claim 22. An important element of the invention recited in claim 10 is the addition of a phenol stabilizer at a concentration of 0.05-0.5% to the extraction reagent. Given the art cited by the Examiner, there is no suggestion to combine the art to further add a phenol stabilizer to the reagent of claim 22

The only manner by which the Examiner would have been able to obtain the invention of claims 10 and 11 (to the extent they depend on claim 22) would have been to use Appellant's application to provide a template for obtaining the reagent. Therefore, the rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure. In view of the remarks above, Appellant submits that the rejection of claims 10 and 11 to the extent that they depend on claim 22 must be reversed.

H. ***Issue H (claims 25-27 and 31)*** Whether an aqueous RNA extraction reagent comprising at least one nonionic detergent (0.1-1.0%), at least one phenol (10-60%), at least one phenol solubilizer (15-55%) and sodium citrate (0.02-0.25 M) is obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution but not sodium citrate as a chelator?

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 25-27 which are directed to an aqueous RNA extraction reagent, comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60%

(wgt/vol), at least one phenol solubilizer at a concentration of 15-55% (vol/vol) and sodium citrate at a concentration of 0.02-0.25 M. Therefore, the comments in section ***VII.A.1*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in sections ***VII.A.2*** are incorporated herein by reference. Appellant further submits that the Examiner has provided no teaching, suggestion or reason why one of ordinary skill in the art would combine Sambrook, Chomczynski and Perlman to obtain an RNA extraction reagent comprising sodium citrate, in particular at a concentration of 0.02-0.25 M, together with at least one non-ionic detergent, at least one phenol and at least one phenol solubilizer.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claims 25-27 and 31. An important element of the invention of claim 25, sodium citrate at 0.02 - 0.25 M, is nowhere to be found in the cited art. Thus, a *prima facie* case of obviousness has not been established.

The rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure. Where else could addition of sodium citrate to the RNA extraction reagent have been found? In view of the remarks above, Appellant submits that the rejection of claims 25-27 and 31 must be reversed.

I. ***Issue I (claims 30, 32 and 33)*** Whether an aqueous RNA extraction reagent comprising octylphenoxy poly(oxyethylene)ethanol at a concentration of about 0.5%, 8-hydroxyquinoline at a concentration of 0.05-0.2%, sodium citrate at a concentration of 0.02-0.25 M, at least one phenol at a

concentration of 10-60% and at least one phenol solubilizer at a concentration of 15-55% are obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution but not citrate as a chelator?

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 30, 32 and 33 which are directed to an aqueous RNA extraction reagent comprising octylphenoxy poly(oxyethylene)ethanol at a concentration of about 0.5%, 8-hydroxyquinoline at a concentration of 0.05-0.2%, sodium citrate at a concentration of 0.02-0.25 M, at least one phenol at a concentration of 10-60%, and at least one phenol solubilizer at a concentration of 15-55%. Therefore, the comments in section ***VII.A.1*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in section ***VII.A.2, VII.C2*** and ***VII.H2*** are also incorporated herein by reference. Appellant further submits that the Examiner has provided no guidance concerning why one would combine the cited art to obtain an RNA extraction reagent octylphenoxy poly(oxyethylene)ethanol at a concentration of about 0.5%, 8-hydroxyquinoline at a concentration of 0.05-0.2%, sodium citrate at a concentration of 0.02-0.25 M, at least one phenol at a concentration of 10-60%, and at least one phenol solubilizer at a concentration of 15-55%. Further, the Examiner has failed to point to anything in the art that would even suggest the combination of the specific non-ionic detergent (octylphenoxy

poly(oxyethylene)ethanol), sodium citrate or any of the other components at the concentrations of claims 30, 32 or 33 to obtain an RNA extraction reagent.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claims 30, 32, or 33. Important elements of the invention, e.g. octylphenoxypoly(oxyethylene) ethanol at a concentration of 0.1-1.0% and sodium citrate at a concentration of 0.02-0.25 M are nowhere to be found in the cited art. Further, nowhere in any of the cited art has the Examiner pointed to the use of octylphenoxypoly(oxyethylene) ethanol at any concentration whatsoever in an RNA extraction reagent. Additionally, the Examiner has failed to point to any motivation or suggestion to combine the cited art to obtain an aqueous RNA extraction reagent comprising octylphenoxy poly(oxyethylene)ethanol, 8-hydroxyquinoline, sodium citrate, at least one phenol and , at least one phenol solubilizer

The rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure. Where else could it have been found? In view of these additional remarks above, Appellant submits that the rejection of claims 30, 32 and 33 must be reversed.

J. ***Issue J (claim 34)*** Whether an aqueous RNA extraction reagent comprising 1) at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one phenol solubilizer at a concentration of 15-55% (vol/vol), or 2) comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one chelator at a concentration of 0.02-0.25 M, said reagent containing a salt of citric acid at a concentration of 0.05-0.2% is obvious in view of Sambrook, which describes an RNA extraction buffer lacking phenol and phenol solubilizer but containing a non-ionic detergent, in view of Chomczynski which describes an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol and a phenol solubilizer but lacking a non-ionic detergent and Perlman which merely describes a chelated phenol solution not the use of a salt of citric acid?

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claim 34 which is directed to an aqueous RNA extraction reagent, comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol), at least one phenol solubilizer at a concentration of 15-55% (vol/vol) or a chelator at a concententration of 0.02-0.2M, said reagent having a salt of citric acid. Therefore, the comments in sections ***VII.A.1*** and ***VII.B.1*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in sections ***VII.A.1*** and ***VII.A.2*** are incorporated herein by reference. Appellant further submits that the Examiner has provided no teaching, suggestion or reason why one of ordinary skill in the art would combine Sambrook, Chomczynski and Perlman to obtain an RNA extraction reagent comprising at least one non-ionic detergent, at least one phenol and at least one phenol solubilizer or chelator said reagent having a salt of citric acid at a concentration of 0.02-0.25 M.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claim 34. An important element of the invention of claim 25, a salt of citric acid, is nowhere to be found in the cited art. Thus, a *prima facie* case of obviousness has not been established.

The rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure. Where else could addition of a salt of citric acid to the RNA

extraction reagent have been found? In view of the remarks above, Appellant submits that the rejection of claim 34 must be reversed.

K. ***Issue K (claims 13-20 to the extent that they depend on claim 21)*** Whether a method for providing cytoplasmic RNA from a sample comprising: (a) mixing said sample containing said cells with an RNA extraction reagent comprising at least one non-ionic detergent (0.1-1.0%), at least one phenol (10-60%) and at least one phenol solubilizer (15-55%) to form a mixture; (b) adding a haloalkane to the mixture and mixing the resulting organic and aqueous phases; (c) separating the organic and aqueous phases; and (d) precipitating cytoplasmic RNA from the aqueous phase obtained in step (c) is *prima facie* obvious in view of Sambrook, Chomczynski and Sambrook, none of which teach the above steps for providing cytoplasmic RNA.

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 13-20 (to the extent they depend on claim 21) which are directed to a method for providing cytoplasmic RNA from a sample using an aqueous RNA extraction reagent, comprising at least one non-ionic detergent at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60% and at least one phenol solubilizer at a concentration of 15-55%. Because the Examiner must first show that the combination of the art suggests the above reagent, the comments in section ***VII.A.1*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in section ***VII.A.2.*** are incorporated herein by reference because the method involves the use of the reagent of claim 21. In order to practice the method of the claims one must first have a reagent comprising at least one non-ionic

detergent, at least one phenol and at least one phenol solubilizer. Therefore, the Examiner must first show that the reagent used in the claimed method is obvious in view of the combination of the cited art. As argued in section ***VII.A.2*** this has not been done. If the reagent is not obvious in view of the cited art, then the rest of the method of RNA isolation using the reagent of claim 21 cannot be obvious.

Appellant further submits that the Examiner has provided no teaching, suggestion or reason why one of ordinary skill in the art would combine the cited art in order to obtain a method having the steps required to practice Appellant's method. Each and every step of a claimed method must be shown to be obvious in view of the cited art in order to render a claimed method obvious. This has not been done.

Claims 13-20 were first rejected over Sambrook, Chomczynski and Perlman in the Office Action mailed May 9, 2000 [Paper No. 11]. No motivation or suggestion to combine the references to obtain the ***method*** of claims 13-20 to the extent that they depended on claim 21, was provided in the May 9, 2000 Office Action or any action thereafter.

Therefore, the Examiner has failed to show that based on the cited art, one would add a haloalkane to a mixture comprising the reagent of claim 21, mix the resulting organic and aqueous phases; separate the organic and aqueous phases; and precipitate the cytoplasmic RNA from the previously obtained aqueous phase.

Perlman, which is merely directed to a chelated phenol solution, provides no method steps for the extraction of RNA or any compounds whatsoever.

Sambrook discusses separate method steps for lysis of cells using an RNA extraction buffer containing NP-40, but not a phenol and a phenol solubilizer, followed by additional steps using a proteinase digestion buffer and protein removal using a phenol-chloroform

mixture. Nowhere does Sambrook suggest a single step of mixing a sample comprising eukaryotic cells with a reagent comprising at least one non-ionic detergent, at least one phenol and at least one phenol solubilizer followed by a step adding a haloalkane to the mixture, separating the organic and aqueous phases and precipitating the RNA from the aqueous layer. While Sambrook does provide a step separating the organic from the aqueous stages, this does ***not*** occur after addition of a reagent having the components as recited in claim 21.

Chomczynski also fails to provide the steps of the claimed method. Chomczynski first mixes a sample with a reagent containing guanidinium thiocyanate, ammonium thiocyanate a buffer, glycerol (3-10%) and a phenol (30-50%). Contrary to Appellant's reagent, this solution lacks both a non-ionic detergent and a phenol solubilizer at 15-55%. Thus, Chomczynski fails to describe mixing a sample with the reagent of claim 21.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claims 13-20 to the extent they depend on claim 21. An important element of the invention, mixing a sample with the reagent of claim 21 is missing from the art.

The rejection can only have been conceived based upon the hindsight provided by Appellant's disclosure. In view of the remarks above, Appellant submits that the rejection of claims13-20 to the extent that they depend on claim 21 must be reversed.

L. ***Issue L (claims 13-20 to the extent that they depend on claim 22)*** Whether a method for providing cytoplasmic RNA from a sample comprising: (a) mixing said sample containing said cells with an RNA extraction reagent comprising at least one non-ionic detergent (0.1-1.0%), at least one phenol (10-60%) and at least one chelator (0.02-0.25 M) to form a mixture; (b) adding a haloalkane to the mixture and mixing the resulting organic and aqueous phases; (c) separating the organic and aqueous phases; and (d) precipitating cytoplasmic RNA from the aqueous phase obtained in step (c)

is *prima facie* obvious in view of Sambrook, Chomczynski and Perlman, none of which teach the above steps for providing cytoplasmic RNA.

1. The Examiner's Obviousness Rejection

The Examiner has not separately rejected claims 13-20 (to the extent they depend on claim 22) which are directed to a method for providing cytoplasmic RNA from a sample using an aqueous RNA extraction reagent, comprising at least one non-ionic detergent at a concentration of 0.1-1.0%, at least one phenol at a concentration of 10-60% and at least one and at least one chelator (0.02-0.25 M). Because the Examiner must first show that the combination of the art suggests the above reagent, the comments in section ***VII.A.1*** are incorporated herein by reference.

2. The Obviousness Rejection is in Error and Must be Reversed

The arguments provided in sections ***VII.A.2., VII.B.2 and VII.I.2*** are incorporated herein by reference because the method involves the use of the reagent of claim 22 with the steps described in the section immediately above. The Examiner must first show that the reagent used in the claimed method was obvious in view of the combination of the cited art. As argued in section ***VII.A.2 and VII.B.2,*** this has not been done. If the reagent is not obvious in view of the cited art, neither can the rest of the method of RNA isolation using the reagent of claim 22 be obvious. It is further added that neither of the methods of Chomczynski or Sambrook suggest a step using a reagent as required for the reagent of claim 22.

Appellant submits that the Examiner has not established a *prima facie* case of obviousness of claims 13-20 to the extent they depend on claim 22. An important element of the invention, mixing a sample with the reagent of claim 22 is missing from the art. Appellant further submits that the Examiner has provided no teaching, suggestion or reason why one of ordinary skill in the art would combine the cited art in an attempt to practice Appellant's method. Each and every step of a claimed method must be shown to be obvious in view of the cited art in order to render a claimed method obvious. This has not been done.

Therefore, the Examiner has failed to show that based on the cited art, one would add a haloalkane to the mixture having the reagent of claim 22, mix the resulting organic and aqueous phases; separate the organic and aqueous phases; and precipitate the cytoplasmic RNA from the previously obtained aqueous phase.

The rejection could only have been conceived based upon the hindsight provided by Appellant's disclosure. In view of the remarks above, Appellant submits that the rejection of claims13-20, to the extent that they depend on claim 22, must be reversed.

VIII. Conclusion.

In summary, the Examiner has failed to provide any objective teaching, suggestion or motivation to combine the cited art to achieve Appellant's invention. In any event, even if the cited art is combined, certain limitations of the claimed invention, as acknowledged by the Examiner, are not taught or suggested by the cited art.

Additionally, the Examiner has ***not*** provided a reasonable basis that justifies a conclusion that the concentration of components in Appellant's invention is obvious where there is no suggestion in the art to use such concentrations. Moreover, the issue is not whether the

cited art reagents could be modified, but rather whether there is a suggestion to make the desired modification. As discussed above, none of the cited art, either alone or in combination suggests the desirability of making any of the claimed RNA extraction reagents or using the same.

Further, the Examiner has improperly used hindsight in making the rejection under 35 U.S.C. § 103.

Accordingly, Appellant respectfully submits that the rejection of all the claims under 35 U.S.C. § 103 as obvious over the combination of Sambrook, Chomczynski and Perlman ***is in error and should be reversed.*** Therefore, all of the claims should be allowed to issue.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Prompt and favorable consideration of this Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

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APPENDIX OF CLAIMS 37 C.F.R. § 1.192(C)(9)

2. An RNA extraction reagent according to claim 21 or 22, wherein said at least one nonionic detergent is present at a concentration of 0.5-0.8% (vol/vol).

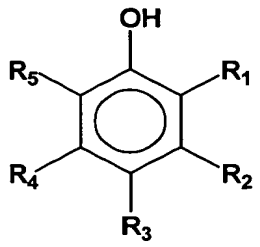
3. An RNA extraction reagent according to claim 2, wherein said nonionic detergent comprises at least one detergent selected from the group consisting of an octylphenoxypoly(oxyethylene)ethanol, an N,N-bis(3-D-gluconamidopropyl)cholamide (BIGCHAP), an decanoyl-N-methylglucamide, an n-decyl α-D-glucopyranoside, an n-decyl β-D-glucopyranoside, an n-decyl β-D-maltopyranoside, a deoxy-BIGCHAP, a digitonin, an n-dodecyl β-D-glucopyranoside, an n-dodecyl α-D-maltoside, an n-dodecyl β-D-maltoside, a heptanoyl-N-methylglucamide, an n-heptyl β-D-glucopyranoside, an N-heptyl β-D-thioglucopyranoside, an n-hexyl β-D-glucopyranoside, a 1-monooleoyl-rac-glycerol, a nonanoyl-N-methylglucamide, an n-nonyl α-D-glucopyranoside, an n-nonyl β-D-glucopyranoside, an octanoyl-N-methylglucamide, an n-octyl α-D-glucopyranoside, an n-octyl β-D-glucopyranoside, an octyl β-D-thiogalactopyranoside, an octyl β-D-thioglucopyranoside, a polyoxyethylene ester, a polyoxyethylene ether, a polyoxyethylene sorbitan ester, TWEEN 20, a sorbitan ester, an n-tetradecyl β-D-maltoside, a triton, a tyloxaapol and an n-undecyl β-D-glucopyranoside.

4 An RNA extraction reagent according to claim 3, wherein said non-ionic detergent is a octylphenoxy poly(oxyethylene)ethanol.

5. An RNA extraction reagent according to claim 4, wherein said octylphenoxy poly(oxyethylene)ethanol is present at a concentration of about 0.5% (vol/vol).

6. An RNA extraction reagent according to claim 22, wherein said chelator is selected from the group consisting of sodium citrate, EDTA, EGTA, a citric acid, a salicylic acid, a salt of a salicylic acid, a tergitol, a phthalic acid, a 2,4-pentanedione, a histidine, a histidinol dihydrochloride, an 8-hydroxyquinoline, an 8-hydroxyquinoline citrate and an *o*-hydroxyquinone.

7. An RNA extraction reagent according to claim 21 or 22, wherein said phenol is a compound according to formula I:



where R_1, R_2, R_3, R_4 and R_5 are each independently selected from H, alkyl, o-alkyl, halo, acyl and hydroxyl.

8. An RNA extraction reagent according to claim 22, further comprising at least one phenol solubilizer at a concentration of 15%-55% (wgt/vol).

9. An RNA extraction reagent according to claim 8, wherein said phenol solubilizer is selected from the group consisting of a monoalcohol, a diol and a polyol.

10. An RNA extraction reagent according to claim 21 or 22, further comprising at least one phenol stabilizer at a concentration of 0.05%-0.2% (wgt/vol).

11. An RNA extraction reagent according to claim 10, wherein said phenol stabilizer is at least one selected from the group consisting of hydroxyquinoline, 8-hydroxyquinoline, 8-hydroxyquinoline citrate, 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-6-chromanol, *p*-hydroxyquinone, *o*-hydroxyquinone, citric acid, or a salt thereof a salt of citric acid, salicylic acid, ascorbic acid, *p*-phenylenediamine and *n*-propylgallate.

13. A method for providing cytoplasmic RNA from a sample comprising eukaryotic cells, said method comprising

(a) mixing said sample containing said cells with an RNA extraction reagent according to claim 21 or 22 to form a mixture;

(b) adding a haloalkane to the mixture and mixing the resulting organic and aqueous phases;

(c) separating the organic and aqueous phases; and

(d) precipitating cytoplasmic RNA from the aqueous phase obtained in step (c).

14. A method according to claim 13, further comprising

(e) recovering the cytoplasmic RNA from the precipitate obtained in step (d).

15. A method according to claim 14, further comprising

(f) isolating mRNA from said cytoplasmic RNA.

16. A method according to claim 14, wherein said sample is derived from a plant or a plant material.

17. A method according to claim 13, wherein said cells are plant cells.

18. A method according to claim 13, wherein said cells are animal cells.

19. A method according to claim 18, wherein said animal cells are mammalian cells.

20. A method according to claim 13, wherein said cells are insect cells.

21. An aqueous RNA extraction reagent, comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one phenol solubilizer at a concentration of 15-55% (vol/vol).

22. An aqueous RNA extraction reagent, comprising at least one nonionic detergent at a concentration of 0.1-1.0% (vol/vol), at least one phenol at a concentration of 10-60% (wgt/vol) and at least one chelator at a concentration of 0.02-0.25 M.

23. An RNA extraction reagent according to claim 22, wherein said chelator is selected from the group consisting of sodium citrate, EDTA, EGTA, tergitols, phthalic acids, 2,4-pentanediones, histidines, histidinol dihydrochlorides, and salts of salicylic acids.

24. An RNA extraction reagent according to claim 23, further comprising at least one phenol stabilizer selected from the group consisting of hydroxyquinoline, 8-hydroxyquinoline, 8-hydroxyquinoline citrate, 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-6-chromanol, *p*-hydroxyquinone, *o*-hydroxyquinone, citric acid, salicylic acid, ascorbic acid, *p*-phenylenediamine and *n*-propylgallate.

25. An RNA extraction reagent according to claim 21, further comprising sodium citrate at a concentration of 0.02 – 0.25 M.

26. An RNA extraction reagent according to claim 25, further comprising at least one phenol stabilizer at a concentration of 0.05 - 0.2% (wgt/vol), said phenol stabilizer selected from the group consisting of hydroxyquinoline, 8-hydroxyquinoline, 8-hydroxyquinoline citrate, 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-6-chromanol, *p*-hydroxyquinone, *o*-hydroxyquinone, citric acid, salicylic acid, ascorbic acid, *p*-phenylenediamine and *n*-propylgallate.

27. An RNA extraction reagent according to claim 25, further comprising 8-hydroxyquinoline at a concentration of 0.05 - 0.2% (wgt/vol).

28. An RNA extraction reagent according to claim 21, further comprising 8-hydroxyquinoline at a concentration of 0.05 - 0.2% (wgt/vol).

29. An RNA extraction reagent according to claim 28, further comprising at least one chelator at a concentration of 0.02 – 0.25 M, said chelator selected from the group consisting of sodium citrate, citric acid, EDTA, EGTA, salicylic acids, salts of salicylic acids, tergitols, phthalic acids, 2,4-pentanediones, histidines, histidinol dihydrochlorides, 8-hydroxyquinoline citrates, and *o*-hydroxyquinones.

30. An RNA extraction reagent according to claim 27, wherein the nonionic detergent is octylphenoxy poly(oxyethylene)ethanol at a concentration of about 0.5% (vol/vol).

31. An RNA extraction reagent according to claim 27, wherein the phenol solubilizer is ethylene glycol.

32. An RNA extraction reagent according to claim 30, wherein the phenol solubilizer is selected from the group consisting of a monoalcohol, a diol and a polyol.

33. An RNA extraction reagent according to claim 30, wherein the phenol solubilizer is ethylene glycol.

34. An RNA extraction reagent according to claim 21 or 22, said reagent containing a salt of citric acid at a concentration of 0.05 - 0.2% (wgt/vol).

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